

1 **Title:** Pharmacodynamics of Isavuconazole in a Rabbit Model of Cryptococcal

2 Meningoencephalitis

3

4 **Running Title:** Pharmacodynamics of Isavuconazole in Cryptococcosis

5

6 **Authors:** Laura L. Kovanda^{a,#}, Charles Giamberardino^b, Laura McEntee^c, Dena

7 L. Toffaletti^b, Kelly S. Franke^b, Andrew Bartuska^b, Gordon Smilnak^b, George C.

8 de Castro^b, Katelyn Ripple^b, William W. Hope^{c,d}, John R. Perfect^b

9

10 **Affiliations:**

11 ^aAstellas Pharma Global Development, Inc., Northbrook, IL, USA

12 ^bDuke University Medical Center, Durham, NC, USA

13 ^cAntimicrobial Pharmacodynamics and Therapeutics, Department of Molecular

14 and Clinical Pharmacology, Institute of Translational Medicine, University of

15 Liverpool, Liverpool Health Partners, Liverpool, UK

16 ^dRoyal Liverpool and Broadgreen University Hospital Trust, Liverpool Health

17 Partners, Liverpool, UK

18

19 **#Corresponding Author:**

20 Laura L Kovanda, PhD

21 Astellas Pharma Global Development, Inc.

22 1 Astellas Way, Northbrook, IL 60062, USA

23 Email: laura.kovanda@astellas.com

24 **ABSTRACT**

25 *Cryptococcus* spp., an important fungal pathogen, is the leading cause of fungal-
26 related mortality in human immunodeficiency virus patients, and new therapeutic
27 options are desperately needed. Isavuconazonium sulfate, a newer triazole
28 antifungal agent, was studied to characterize the exposure–response relationship
29 in a rabbit model of cryptococcal meningoencephalitis. Isavuconazonium sulfate
30 treatment was compared with fluconazole and untreated controls. Fungal burden
31 in the cerebrospinal fluid was measured serially over time, while yeast
32 concentrations in the brain and the eye (aqueous humor) were determined at the
33 end of therapy. The exposure impact of isavuconazonium sulfate dosing in the
34 rabbit was linked using mathematical modeling. Similar significant reductions in
35 fungal burden in the brain and cerebrospinal fluid were observed with
36 isavuconazonium sulfate and fluconazole treatment compared with untreated
37 controls. No dose-dependent response was demonstrated with isavuconazonium
38 sulfate treatment in this study. The treatment of cryptococcal
39 meningoencephalitis with isavuconazonium sulfate was similar to that with
40 fluconazole. Dose-dependent reductions in yeast over time were not
41 demonstrated, which limited our ability to estimate the pharmacodynamic target.
42 Further non-clinical and clinical studies are needed in order to characterize the
43 extent of the exposure–response relationship in cryptococcal
44 meningoencephalitis. However, this study suggests isavuconazonium sulfate, like
45 fluconazole, could be beneficial in the setting of consolidation and maintenance

- 46 therapy, rather than induction monotherapy with high burden cryptococcal
- 47 meningoencephalitis.

48 INTRODUCTION

49 Infections caused by *Cryptococcus neoformans* and *C. gattii* are associated
50 with excessive morbidity and mortality in patients with and without HIV. *C.*
51 *neoformans* is the leading cause of fungal-related mortality in HIV patients,
52 especially in sub-Saharan Africa where the HIV/AIDS epidemic is persistent (1).
53 At the peak of the HIV epidemic, approximately 1 million cases of cryptococcosis
54 were reported annually in patients with AIDS (1). In the developed world, cases
55 of cryptococcosis declined over the same time period as the use of antiretroviral
56 therapy increased. In contrast, the prevalence remained high in low to middle
57 income countries (2). In the United States, it is estimated that nearly 3,400
58 hospitalizations occur yearly due to cryptococcal meningitis (3). Direct
59 hospitalization-associated costs for cryptococcal meningitis amounted to
60 approximately US\$54 million in 2009 (3).

61 Treatment of cryptococcal meningitis typically includes induction therapy with
62 amphotericin B deoxycholate (0.7–1.0 mg/kg per day) or AmBisome (3–4 mg/kg
63 per day intravenously) plus flucytosine (100 mg/kg per day orally) in four divided
64 dosages for 1 week, followed by consolidation therapy with fluconazole (400 mg
65 [6 mg/kg] per day orally) for a minimum of 8 weeks (4). In some areas of the
66 world, fluconazole may be the only available agent for induction therapy (4). High
67 baseline cerebrospinal fluid (CSF) fungal burden, altered mental status, older
68 age, and high peripheral white blood counts predict acute 2-week mortality.
69 Furthermore, improved outcomes were associated with amphotericin-based
70 treatment and prompt immune reconstitution with antiretroviral therapy (5).

71 Cryptococcal infection generally begins in the lungs, although meningitis is
72 the most frequently encountered clinical manifestation of cryptococcosis among
73 those with advanced immunosuppression. The disease is more properly
74 characterized as “meningoencephalitis” rather than meningitis since the brain
75 parenchyma is almost invariably involved on histologic examination (6). This
76 understanding is critical when determining a successful treatment strategy, as
77 central nervous system (CNS) penetration into the brain tissue as well as into the
78 CSF is vital to improve outcome.

79 Isavuconazonium sulfate, the water-soluble prodrug of the broad-spectrum
80 triazole antifungal agent isavuconazole, is approved by the U.S. FDA for the
81 treatment of invasive aspergillosis and invasive mucormycosis, and by the
82 European Medicines Agency for the treatment of invasive aspergillosis, and
83 invasive mucormycosis in patients for whom amphotericin B is inappropriate (7,
84 8) (9). Isavuconazole MIC values against *Cryptococcus spp.* range from 0.008
85 mg/L to 0.5 mg/L with an overall modal MIC of 0.03 mg/L in a collection of more
86 than 800 *Cryptococcus neoformans* isolates (10).

87 To further support an understanding of the effectiveness of isavuconazonium
88 sulfate against cryptococcal infections, particularly in the CNS, we characterized
89 the exposure–response relationship of isavuconazonium sulfate in a well-
90 established rabbit model of cryptococcal meningoencephalitis caused by
91 *Cryptococcus neoformans*.

92

93 RESULTS

94 **Cryptococcus neoformans (H99) MIC values.** The geometric mean
95 isavuconazole MIC value for the H99 strain of *Cryptococcus neoformans* was
96 0.008 mg/L for isavuconazole and 1 mg/L for fluconazole. Yeast grown from
97 treatment Day 8 of the isavuconazonium sulfate treatment animals showed
98 similar MIC values.

99 **Animal model.** Significant reductions in fungal burden were seen in the brain
100 following administration of isavuconazonium sulfate doses of 83 mg/kg
101 (equivalent to 45 mg/kg of isavuconazole) and 111.8 mg/kg (equivalent to 60
102 mg/kg of isavuconazole). These were comparable to 80 mg/kg of fluconazole.
103 Both isavuconazonium sulfate and fluconazole treatments resulted in significant
104 decreases in fungal burden in the brain compared with untreated controls ($P =$
105 0.0003; $P = 0.0002$; $P = 0.0034$, one-way analysis of variance (ANOVA), Holm–
106 Šidák’s multiple comparisons test) (**Fig. 1a**). Treatment with isavuconazonium
107 sulfate and fluconazole resulted in significant reductions in the CSF fungal
108 burden of yeast over time compared with controls ($P < 0.0001$, two-way ANOVA,
109 Tukey’s multiple comparisons test) (**Fig. 1b**). Investigation of the residual fungal
110 burden in the eye (aqueous humor) were extremely variable and were not
111 considered reliable; therefore, the results are not reported here.

112 **Pharmacokinetics.** Mean isavuconazole concentrations in the brain tissue
113 (cerebrum, cerebellum, meninges) at the end of the experiment were similar
114 between the isavuconazonium sulfate dose groups (**Table 1**). The mean ratio of
115 brain to plasma isavuconazole concentrations in rabbits was 0.69 and 0.42 for
116 the 83.8 mg/kg and 111.8 mg/kg doses, respectively. As with the brain tissue, the

117 mean isavuconazole concentrations in the CSF did not increase with increasing
118 dose (**Table 1**). The mean ratio of CSF to plasma ratio was 0.044 and 0.019 for
119 the 83.8 mg/kg and 111.8 mg/kg doses, respectively (**Table 1**).

120 **Pharmacokinetic/pharmacodynamic modeling.**

121 Pharmacokinetic/pharmacodynamic modeling using the brain isavuconazole
122 concentration data and fungal burden in the CNS was not possible because of
123 the lack of temporal data for measures. Therefore, the
124 pharmacokinetic/pharmacodynamic model focused on a link between plasma
125 and CSF isavuconazole concentrations and CSF fungal burden over time.

126 The fit of the model to the plasma and CSF isavuconazole concentration data
127 and yeast colony-forming units in the CSF over time was acceptable based on
128 visual inspection of the median observed-versus-predicted plots. A linear
129 regression had a coefficient of determination (r^2) of 0.841 (slope = 0.951), 0.745
130 (slope = 0.958), and 0.692 (slope = 0.853) after the Bayesian step for the
131 isavuconazole concentrations in the plasma, CSF and CSF fungal burden,
132 respectively (**Fig. 2a, b and c**). The estimates of bias and imprecision were also
133 acceptable (plasma: -0.122 and 1.19; CSF: -0.0246 and 2.02; CSF CFU:
134 0.00863 and 0.0207). The observed-versus-predicted plots using the mean
135 parameter values were similar (data not shown). The mean parameter estimates
136 are included in **Table 2**.

137 The median Bayesian posterior pharmacokinetic parameters from the
138 pharmacokinetic/pharmacodynamic model were used to estimate the area under
139 the concentration-time curve from 0 h to 24 h (AUC_{0-24}) for the plasma and CSF,

140 as well as the area under the CSF fungal burden-versus-time relationship (**Table**
141 **3**). Although there was a nearly twofold increase in the plasma isavuconazole
142 exposures, drug exposure in the CSF did not increase. Not surprisingly, this lack
143 of increase in exposure by dose in the CSF compartment resulted in minimal
144 differences in response by dose (**Table 3**). This lack of change in exposure and
145 effect by dose resulted in the inability to explore and characterize the
146 pharmacodynamic target (50% effective concentration) for response in the
147 inhibitory sigmoid Emax model with this dataset.

148

149 **DISCUSSION**

150 Treatment options for cryptococcal infections are limited and the available
151 antifungal agents have not changed substantially in the last ~20 years. It was not
152 until recently that characterization of the exposure–response relationship with
153 animal models and bridging studies became available to guide dosing in clinical
154 studies (11, 12). These efforts led to modifications in sequence and duration of
155 induction therapy, as well as changes to dosage of the three recommended
156 agents: amphotericin B, flucytosine, and fluconazole (4). Access to all three
157 medications in countries with limited resources—where the incidence of the
158 infection is highest—is inconsistent (2). Fluconazole is the only agent available in
159 some of these countries. In the current study, isavuconazonium sulfate
160 demonstrated significant reductions in CSF and brain fungal burden in rabbits
161 infected with *Cryptococcus neoformans* (H99) compared with untreated rabbits,

162 and similar to fluconazole. However, a dose-dependent response was not
163 demonstrated for isavuconazole for the dosages used in this study.

164 Isavuconazonium sulfate regimens used to treat invasive mold infections
165 result in total drug plasma AUCs of approximately 90 mg*h/L (13). There is
166 limited data on CNS penetration. One report that details the response of patients
167 receiving isavuconazonium sulfate dosages ranging from 372 mg (equivalent to
168 200 mg isavuconazole) to 1,116 mg/day (equivalent to 600 mg isavuconazole) is
169 available (14). CSF concentrations measured during treatment are summarized
170 in **Table 4**. The ratio of CSF to plasma was consistently 0.008:0.011 and did not
171 increase with increasing dose. If one assumes similar CSF penetration from
172 rabbits to humans, the CSF concentrations achieved with doses above 372
173 mg/day are closer to those concentrations observed in the rabbits. Limited data
174 are available on human brain concentrations of isavuconazole reporting a brain
175 to plasma ratio from one patient of 0.9 (15).

176 Given that a significant proportion of the clinical impact of cryptococcal
177 disease in humans relates to involvement of the brain, the extent of penetration
178 into the cerebral parenchyma is important. Data from radiolabeled
179 isavuconazonium sulfate administered to rats show a brain to plasma
180 concentration ratio of approximately 1.8:1 (16). A similar ratio has been reported
181 from infected mice after receiving isavuconazonium sulfate for 14 days (17). An
182 *in vivo* model of CNS disease has been conducted with isavuconazonium sulfate
183 in mice infected with two strains of *Cryptococcus neoformans* (USC 1597 and
184 H99) (17). Significant improvements in survival and reductions in fungal burden

185 in the cerebrum were observed with isavuconazonium sulfate treatment
186 compared with controls and the effects were similar to treatment with
187 fluconazole.

188 In the clinical setting, nine patients diagnosed with cryptococcosis were
189 treated with isavuconazonium sulfate in an open-label study (VITAL study) (18).
190 Eight patients were alive through ≥ 84 days and six were considered to be
191 treatment successes.

192 As some yeasts persisted in the subarachnoid space for 12 days after
193 treatment, we checked several colonies from the rabbit CSF after 8 days of
194 treatment and there was no change in their original MICs, suggesting no
195 evidence of direct selection for drug-resistant isolates (data not shown). Yeasts
196 showed a non-significant reduction in the eyes following treatment with both
197 isavuconazonium sulfate and fluconazole compared with untreated controls.

198 The current study has several limitations. First, there was insufficient data
199 available to understand the temporal drug exposure in the cerebrum and eyes,
200 eliminating an ability to characterize the exposure–response relationships in
201 these tissues. Only a single yeast strain was studied in the rabbit model, which
202 limits an ability to understand the potential impact of pharmacodynamic variability
203 on the clinical utility of isavuconazonium sulfate for cryptococcal meningitis.
204 Despite these limitations, treatment with isavuconazonium sulfate and
205 fluconazole provided similar significant reductions in cryptococcal burden in both
206 brain and CSF. Additional preclinical and clinical studies are required to further

207 define the clinical utility and potential indications of isavuconazonium sulfate for
208 cryptococcal meningoencephalitis.

209

210 MATERIALS AND METHODS

211 **Organism.** A clinical strain of *Cryptococcus neoformans* (H99) was used.
212 H99 yeasts were stored at -80°C in 25% glycerol. Isavuconazole and
213 fluconazole MICs were tested using the CLSI M27-A3 macrodilution
214 methodology. Briefly, RPMI 1640 broth, using an inoculum of 0.5×10^3 to $2.5 \times$
215 10^3 CFU/mL, was incubated for 72 h at 35°C . The endpoint for determination of
216 the MIC was defined as $\leq 50\%$ reduction in growth relative to the growth of control
217 yeasts without drug exposure.

218 **Rabbit model of cryptococcal meningitis.** Male New Zealand white rabbits
219 weighing 2–3 kg were used; this model has been previously described (12). The
220 Duke University IACUC approved the protocol prior to study initiation.

221 Rabbits were individually housed and maintained with water and standard
222 rabbit feed *ad libitum*. Immunosuppression was induced using hydrocortisone
223 acetate 5 mg/kg by intramuscular injection daily starting on Day -1 and
224 continuing through Day 13 post-infection. Rabbits were given hand-fed
225 vegetables and supplemental subcutaneous fluids throughout the study. They
226 were weighed daily throughout the study. Rabbits were sedated with an
227 intramuscular injection of a mixture of ketamine (30 mg/kg) and xylazine (3
228 mg/kg) prior to intracisternal inoculation and cisternal taps. Intravenous (i.v.)
229 yohimbine was used to reverse the sedation, and rabbits were recovered under

230 supplemental heat with continuous monitoring. Prior to euthanasia, rabbits were
231 sedated with ketamine and xylazine (40 mg/kg and 5 mg/kg, respectively).

232 **Pharmacokinetic blood collections.** Collection was performed on Day 8
233 post-infection. For pharmacokinetic blood draws, rabbits were sedated with
234 acepromazine, 1 mg/kg, and a catheter was placed in the ear artery and sutured
235 in place. For each blood draw, the catheter was flushed with heparinized saline.
236 A pre-sample was then collected and discarded to remove any residual heparin
237 and then the sample was collected from the catheter. Blood samples were taken
238 in EDTA plasma separator tubes at the indicated timepoints post-drug
239 administration. Plasma was isolated by centrifugation and stored at -80°C prior
240 to testing.

241 **Pharmacokinetic Tissue collections and processing.** Brain and
242 meningeal tissue was collected at the end of the experiment. Tissue samples
243 were weighed and transferred to a homogenization tube. The weight was
244 multiplied by 3 for brain and 5 for meninges to adjust the volume of the
245 reconstitution solvent to the samples. Tissues were homogenized for 30 seconds
246 at 4 m/s and repeated as necessary.

247 **Inoculation.** *C. neoformans* isolate, strain H99, was grown at 30°C for 2–3
248 days on yeast extract-peptone-dextrose (YPD) plates. A single colony was
249 selected and a 25 mL YPD broth culture was initiated and grown for 2 days at
250 30°C in a shaker incubator. The organisms were washed twice in phosphate-
251 buffered saline (PBS) and diluted to 3.9×10^6 cells/mL in PBS. In previous
252 studies, we used 1×10^8 cells of *C. neoformans* per inoculation, but a pilot study

253 indicated that a lower inoculum, 1×10^6 cells, was required for a 14-day study. A
254 volume of 0.3 mL of inoculum was loaded into 3 mL syringes with 25-gauge
255 needles. The yeast inoculum was administered by injecting 0.3 mL
256 intracisternally on Day -2 before dosing. The rabbits were injected with
257 yohimbine, 0.2 mg/kg i.v. As mentioned above, rabbits were given supplement
258 subcutaneous fluids during recovery from sedation.

259 **Treatment.** Isavuconazole was administered as a water-soluble prodrug,
260 isavuconazonium sulfate, via oral gavage. The drug was stored in sterilized glass
261 vials at -20°C . Prior to dosing each day, an aliquot was removed and a volume
262 of pH 4 USP-grade water was added to the aliquot to yield a solution of 60
263 mg/mL of prodrug. Prodrug doses included 83.8 mg/kg and 111.8 mg/kg once
264 daily, which delivered 45 mg/kg and 60 mg/kg, respectively, of isavuconazole to
265 the animal. Fluconazole was purchased as an oral suspension and administered
266 i.v. at a dose of 80 mg/kg per day. It was reconstituted with USP water to a final
267 concentration of 40 mg/mL. Untreated controls received saline. Drug dosing
268 began 48 hours post-inoculation and continued for 12 consecutive days.

269 **Fungal burden of tissue.** All rabbits were humanly sacrificed after the final
270 CSF collection, either on Day 10 or at the time of sacrifice. Brains were removed
271 and then dissected sagittally for quantitative cultures and drug-level analysis.
272 Fungal burden was measured in brain (cut into three sections) and eyes (vitreous
273 humor and aqueous humor). Tissue was placed in pre-weighed tubes containing
274 1 mL of PBS and then weighed again to find the net weight of the tissue. Tissue
275 was homogenized using a Pro200 (Pro Scientific, Oxford, CT, USA) in a

276 biological safety cabinet. The tissue homogenates were 10-fold serially diluted,
277 and then plated on YPD with chloramphenicol agar. Cultures were grown for 3
278 days at 30°C. CFUs were quantified as the CFU/g of tissue. The fungal burden
279 within the CSF from the subarachnoid space was measured from serial samples
280 taken at 48, 96, 144, 192, and 240 h post-inoculation via an intracisternal tap with
281 a 3 mL syringe and a 25-gauge needle, serially diluted (1:10), in PBS in 1 mL
282 and plated on YPD with chloramphenicol agar, and incubated at 30°C for 2–3
283 days, then viable yeast colonies were counted. Calculations for CFU/mL were as
284 follows: (CFU counted × dilution)/0.1 mL. The remaining CSF was centrifuged,
285 and the supernatant stored at –80°C.

286 **Isavuconazole bioanalytical assay.** Isavuconazole concentrations in rabbit
287 plasma, CSF and brain were measured using a Thermo Fisher Scientific
288 Vanquish (Waltham, MA, USA) ultraperformance liquid chromatography coupled
289 with a Thermo Fisher Scientific Q Exactive™ Focus (Waltham, MA, USA) mass
290 spectrometer. The method used an ACE 5 C18-AR 50 × 3.0 mm (supplier:
291 Hichrom Ltd, Reading, UK) and a 3 µL injection volume. A standard curve
292 encompassing 15 to 40,000 ng/mL was constructed from stock solutions of
293 isavuconazole 1 mg/mL in dimethyl sulfoxide. Chromatographic separation was
294 achieved using gradient conditions starting with 35:65 (0.1% formic acid in water
295 as mobile phase A, and 0.1% formic acid in acetonitrile as mobile phase B).
296 Mobile phase B was increased to 95% from 0.2 to 1.0 min and held for 1.6 min.
297 At 1.7 min mobile phase B was reduced to 65%. This was all at a flow rate of 0.6
298 mL/min. Isavuconazole was detected using the exact mass in positive ion mode

(438.1195). Isavuconazole eluted after 1.0 min. The limit of detection was 15 ng/mL and the assay was linear over the concentration range 15 to 40,000 ng/mL. The quality control intra- and inter-day accuracy was 98.7–104.6%. The quality control coefficient of variation percentage was 1.1–8.4%.

Pharmacokinetic/pharmacodynamic mathematical modeling. Population pharmacokinetic/pharmacodynamic mathematical modeling was performed using non-parametric estimation, Pmetrics™ (v. 1.5.2, University of Southern California, Los Angeles, CA, USA) (19) and fitted to the rabbit plasma and CSF isavuconazole concentration data, and the CSF CFUs. Data were weighted by the inverse of the estimated assay variance. The linked pharmacokinetic/pharmacodynamic model was constructed using the following set of differential equations:

$$\text{Eq. 1} \quad \frac{dX(1)}{dt} = -Ka \cdot X(1)$$

$$\text{Eq. 2} \quad \frac{dX(2)}{dt} = Ka \cdot X(1) - \left(\left(\frac{Cl}{V} \right) \cdot X(2) \right) - Kcp \cdot X(2) + Kpc \cdot X(4) - Kcm \cdot X(2) + Kmc \cdot X(3)$$

$$\text{Eq. 3} \quad \frac{dX(3)}{dt} = Kcm \cdot X(2) - Kmc \cdot X(3)$$

$$\text{Eq. 4} \quad \frac{dX(4)}{dt} = Kcp \cdot X(2) - Kpc \cdot X(4)$$

$$\text{Eq. 5:} \quad \frac{dX(5)}{dt} = Kgmax \cdot \left(1 - \left(\frac{\left(\frac{X(3)}{V} \right)^{Hg}}{c50g^{Hg} + \left(\frac{X(3)}{V} \right)^{Hg}} \right) \right) \cdot X(5) \cdot$$

$$\left(1 - \left(\frac{X(5)}{popmax} \right) \right) - kkmax \cdot X(5) \cdot \left(\frac{\left(\frac{X(3)}{V} \right)^{Hk}}{c50g^{Hk} + \left(\frac{X(3)}{V} \right)^{Hk}} \right)$$

318 The first four equations describe the pharmacokinetics of the isavuconazole,
319 (compartment 1, theoretical absorptive compartment for oral administration; 2,
320 central compartment; 3, CSF compartment; and 4, peripheral compartment). Cl is
321 the clearance and defined as the amount of drug being cleared from the central
322 compartment over time, and V is the volume of the central compartment. Ka is
323 the first order absorption constant; Kcp, Kpc, Kcm, and Kmc are the rate
324 constants describing the flow of drug to and from compartments 2, 3, and 4.
325 Equation 5 describes the rate of change of the *Cryptococcus* yeasts as \log_{10}
326 CFU/mL of CSF. Kgmax represents the maximum rate of growth; Hg is the slope
327 function for growth; C50g is the amount of drug where there is half maximal
328 growth; popmax is the theoretical maximum density of yeasts in the CSF; kkmax
329 is the maximum rate of growth inhibition; C50k is the amount of drug where there
330 is half maximal growth inhibition; and Hk is the slope function for growth
331 inhibition.

332 The final model was assessed by: a visual inspection of the observed-versus-
333 predicted concentration values plotted over time after the Bayesian step; the
334 coefficient of determination (r^2) from the linear regression of the observed-versus-
335 predicted values; and evaluation of the estimated bias (mean weighted error) and
336 precision (adjusted mean weighted squared error). After fitting the model to the
337 pharmacokinetic/pharmacodynamic data, the Bayesian posterior estimates for
338 each rabbit were used to estimate the plasma and CSF concentration-time
339 profiles for isavuconazole and the change in yeast CFUs over time in the CSF for
340 each rabbit. CSF AUCs and the area under the \log_{10} CFUs/mL in the

341 *Cryptococcus* spp. in the CSF over time for each rabbit were calculated by
342 integration from the simulated concentration-time profiles (on the last day of
343 dosing for plasma) in Pmetrics.
344 Statistical comparisons were performed in GraphPad Prism version 8.0
345 (GraphPad Software, San Diego, CA, USA).

346 **ACKNOWLEDGMENTS**

347 Isavuconazonium sulfate has been co-developed by Astellas Pharma Global
348 Development, Inc. and Basilea Pharmaceutica International Ltd. This work was
349 supported by Astellas Pharma Global Development, Inc. Northbrook, IL, USA.

350

351 L.L.K. is an employee of Astellas Pharma Global Development, Inc.

352

353 J.R.P. is supported in research by Public Service Grants AI73896, AI93257, and
354 AI04533; and has received research grants and served as an advisory consultant
355 for Pfizer, Astellas, Scynexis, Matinas, Viamet, F2G, Cidara, Merck, Amplyx, and
356 Vical.

357

358 W.H. holds or has recently held research grants with F2G, AiCuris, Astellas
359 Pharma, Spero Therapeutics, Matinas Biosciences, Antabio, Amplyx, Allecra,
360 Bugworks, NAEJA-RGM, the AMR Centre, and Pfizer; and personal fees in his
361 capacity as a consultant for F2G, Amplyx, Ausperix, Spero Therapeutics and
362 BLC/TAZ. He holds awards from the Medical Research Council, National Institute
363 of Health Research, FDA and the European Commission; and is an Ordinary
364 Council Member for the British Society of Antimicrobial Chemotherapy.

365

366 All other authors have no conflicts of interest to declare.

367

368

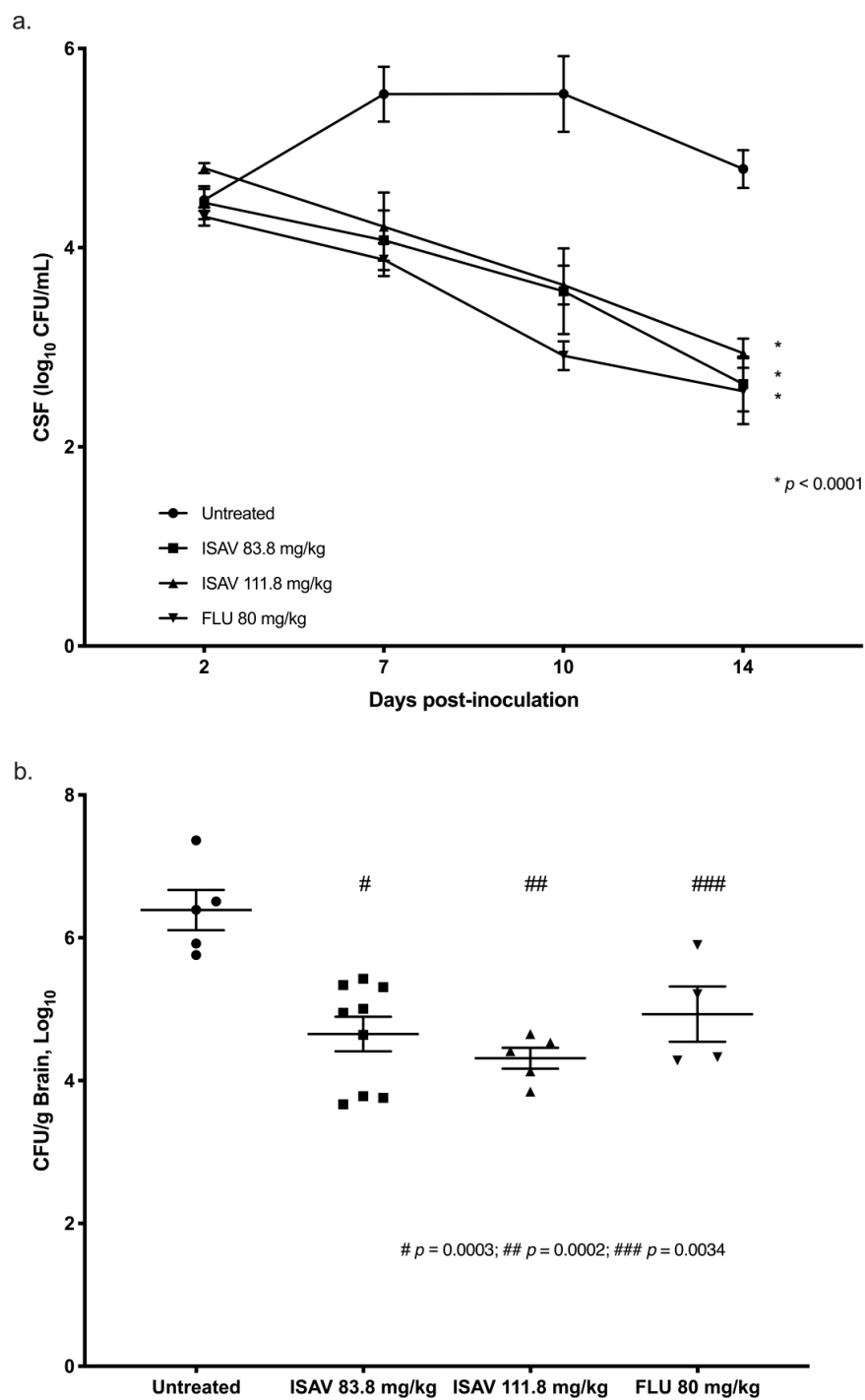
369 REFERENCES

- 370 1. Park B, Wannemuehler K, Marston B, Govender N, Pappas P, Chiller T.
371 2009. Estimation of the current global burden of cryptococcal meningitis
372 among persons living with HIV/AIDS. *AIDS* 23:525-530.
- 373 2. Rajasingham R, Smith R, Park B, Jarvis J, Govender N, Chiller T, Denning
374 D, Loyse A, Boulware D. 2017. Global burden of disease of HIV-
375 associated cryptococcal meningitis: an updated analysis. *Lancet Infect Dis*
376 17:873–881.
- 377 3. Pyrgos V, Seitz A, Steiner C, Prevots D, Williamson P. 2013.
378 Epidemiology of cryptococcal meningitis in the US: 1997-2009. *PLoS One*
379 8:1-6.
- 380 4. World Health Organization. 2018. Guidelines for the Diagnosis, Prevention
381 and Management of Cryptococcal Disease in HIV-Infected Adults,
382 Adolescents and Children: Supplement to the 2016 Consolidated
383 Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing
384 HIV Infection. WHO Guidelines Approved by the Guidelines Review
385 Committee. World Health Organization, Geneva.
- 386 5. Jarvis JN, Bicanic T, Loyse A, Namarika D, Jackson A, Nussbaum JC,
387 Longley N, Muzooru C, Phulusa J, Taseera K, Kanyembe C, Wilson D,
388 Hosseinipour MC, Brouwer AE, Limmathurotsakul D, White N, van der
389 Horst C, Wood R, Meintjes G, Bradley J, Jaffar S, Harrison T. 2014.
390 Determinants of mortality in a combined cohort of 501 patients with HIV-
391 associated cryptococcal meningitis: implications for improving outcomes.
392 *Clin Infect Dis* 58:736–745.
- 393 6. Lee S, Dickson D, Casadevall A. 1996. Pathology of cryptococcal
394 meningoencephalitis: analysis of 27 patients with pathogenetic
395 implications. *Hum Pathol* 27:839-847.
- 396 7. Astellas Pharma US Inc. 2015. CRESEMBA™ (isavuconazonium sulfate)
397 prescribing information. Available at:
398 <http://www.astellas.us/docs/cresemba.pdf>. accessed on March 08, 2019).
- 399 8. European Medicines Agency. 2015. Cresemba (isavuconazonium sulfate)
400 Product Information. Available at:
401 http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/002734/human_med_001907.jsp&mid=WC0b01ac058001d124.
402 (accessed on March 08, 2019).
- 403 9. Thompson GR, 3rd, Wiederhold NP. 2010. Isavuconazole: a
404 comprehensive review of spectrum of activity of a new triazole.
405 *Mycopathologia* 170:291-313.
- 406 10. Espinel-Ingroff A, Chowdhary A, Gonzalez GM, Guinea J, Hagen F, Meis
407 JF, Thompson 3rd GR, Turnidge J. 2015. Multicenter study of
408 isavuconazole MIC distributions and epidemiological cutoff values for the
409 *Cryptococcus neoformans*- *Cryptococcus gattii* species complex using the
410 CLSI M27-A3 broth microdilution method. *Antimicrob Agents and*
411 *Chemother* 59:666-668.
- 412

- 413 11. Sudan A, Livermore J, Howard SJ, Al-Nakeeb Z, Sharp A, Goodwin J,
414 Gregson L, Warn PA, Felton TW, Perfect JR, Harrison TS, Hope WW.
415 2013. Pharmacokinetics and pharmacodynamics of fluconazole for
416 cryptococcal meningoencephalitis: implications for antifungal therapy and
417 in vitro susceptibility breakpoints. *Antimicrob Agents Chemother* 57:2793-
418 2800.
- 419 12. Livermore J, Howard SJ, Sharp AD, Goodwin J, Gregson L, Felton T,
420 Schwartz JA, Walker C, Moser B, Muller W, Harrison TS, Perfect JR,
421 Hope WW. 2014. Efficacy of an abbreviated induction regimen of
422 amphotericin B deoxycholate for cryptococcal meningoencephalitis: 3
423 days of therapy is equivalent to 14 days. *MBio* 5:e00725-00713.
- 424 13. Kovanda L, Desai A, Lu Q, Townsend R, Akhtar S, Bonate P, Hope W.
425 2016. Isavuconazole population pharmacokinetic analysis using non-
426 parametric estimation in patients with invasive fungal disease: results from
427 the VITAL Study. *Antimicrobial Agents and Chemotherapy* 60:4568–4576.
- 428 14. Everson N, Smith J, Garner D. 2015. Successful treatment of
429 contaminated epidural steroid associated fungal meningitis with
430 isavuconazole, abstr P0231. 25th Eur Cong Clin Microbiol Infect Dis,
431 European Society of Clinical Microbiology and Infectious Diseases,
432 Copenhagen, Denmark.
- 433 15. Schmitt-Hoffmann A-H, Kato K, Townsend R, Potchoiba M, Hope WW,
434 Andes D, Spickermann J, Schneidkraut M. 2017. Tissue distribution and
435 elimination of isavuconazole following single and repeat oral dose
436 administration of isavuconazonium sulfate to rats. *Antimicrob Agents*
437 *Chemother* 61:pii.
- 438 16. Wiederhold NP, Kovanda L, Najvar LK, Bocanegra R, Olivo M, Kirkpatrick
439 WR, Patterson TF. 2016. Isavuconazole is effective for the treatment of
440 experimental cryptococcal meningitis. *Antimicrob Agents Chemother*
441 60:5600-5603.
- 442 17. Thompson, GR, 3rd, Rendon A, Ribeiro Dos Santos R, Queiroz-Telles F,
443 Ostrosky-Zeichner L, Azie N, Maher R, Lee M, Kovanda L, Engelhardt M,
444 Vazquez JA, Cornely OA, Perfect JR. 2016. Isavuconazole treatment of
445 cryptococcosis and dimorphic mycoses. *Clin Infect Dis* 63:356-362.
- 446 18. Neely MN, van Guilder MG, Yamada WM, Schumitzky A, Jelliffe RW.
447 2012. Accurate detection of outliers and subpopulations with Pmetrics, a
448 nonparametric and parametric pharmacometric modeling and simulation
449 package for R. *Ther Drug Monit* 34:467-476.
- 450
- 451

452 **FIGURES**

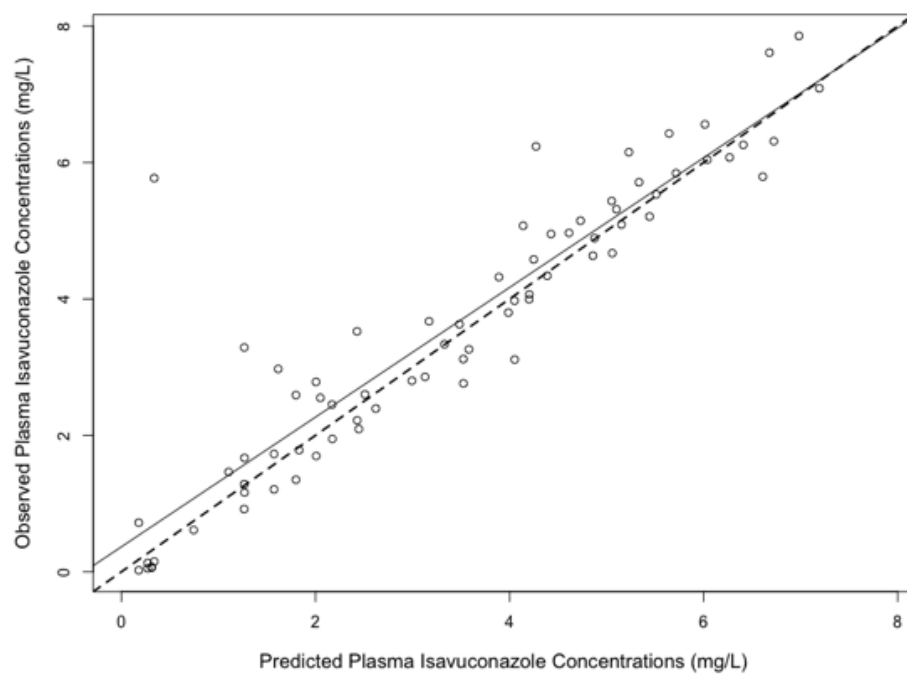
453 **FIG 1 a-b** Change in fungal burden in the rabbit. **(a)** Brain: All treatment groups
454 (isavuconazonium sulfate 83.8 mg/kg and 111.8 mg/kg, and fluconazole 80
455 mg/kg) resulted in significant reductions in log₁₀ CFU/g in the brain at the end of
456 treatment compared with untreated rabbits ($P = 0.0003$; $P = 0.0002$; $P = 0.0034$,
457 one-way ANOVA, Holm–Šidák’s multiple comparisons test). There was no
458 statistical difference between either isavuconazonium sulfate treatment group
459 versus fluconazole ($P > 0.05$). **(b)** CSF: Significant changes in log₁₀ CFUs/mL
460 over time in CSF were demonstrated for all treatment groups (isavuconazonium
461 sulfate 83.8 mg/kg and 111.8 mg/kg, and fluconazole 80 mg/kg) versus untreated
462 rabbits ($P < 0.0001$; Tukey’s multiple comparison test). Note: Not all animals
463 survived to Day 14.
464 CSF, cerebrospinal fluid; ISAV, isavuconazonium sulfate; FLU, fluconazole.



465

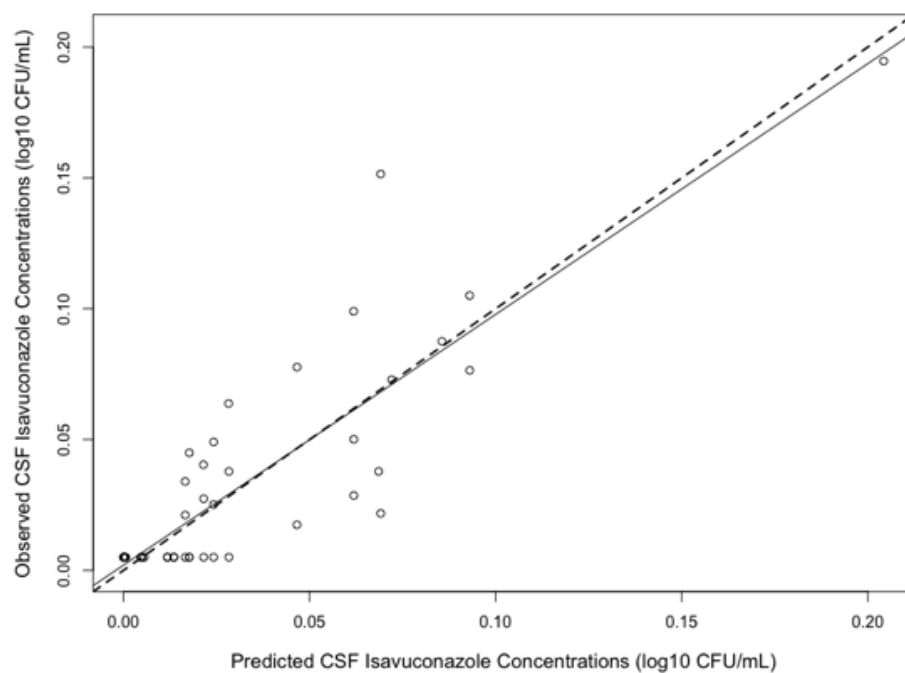
22

467 **FIG 2 (a)** Observed versus median posterior predicted isavuconazole plasma
468 concentrations (mg/L) from the final model after the Bayesian step ($r^2 = 0.841$,
469 slope = 0.951 [95% CI 0.853 to 1.050], intercept = 0.366 [95% CI -0.018 to
470 0.751]). **(b)** Observed versus median posterior predicted isavuconazole CSF
471 concentrations (mg/L) from the final model after the Bayesian step ($r^2 = 0.745$,
472 slope = 0.958 [95% CI 0.79 to 1.13], intercept = 0.00198 [95% CI -0.00617 to
473 0.01010]). **(c)** Observed versus median posterior predicted \log_{10} CFU/mL of CSF
474 from the final model after the Bayesian step ($r^2 = 0.692$, slope = 0.853 [95% CI
475 0.69 to 1.02], intercept = 0.577 [95% CI -0.0697 to 1.22]). Dotted line is line of
476 unity where observed equals predicted concentrations.
477 CSF, cerebrospinal fluid.
478

479 **FIG 2a**

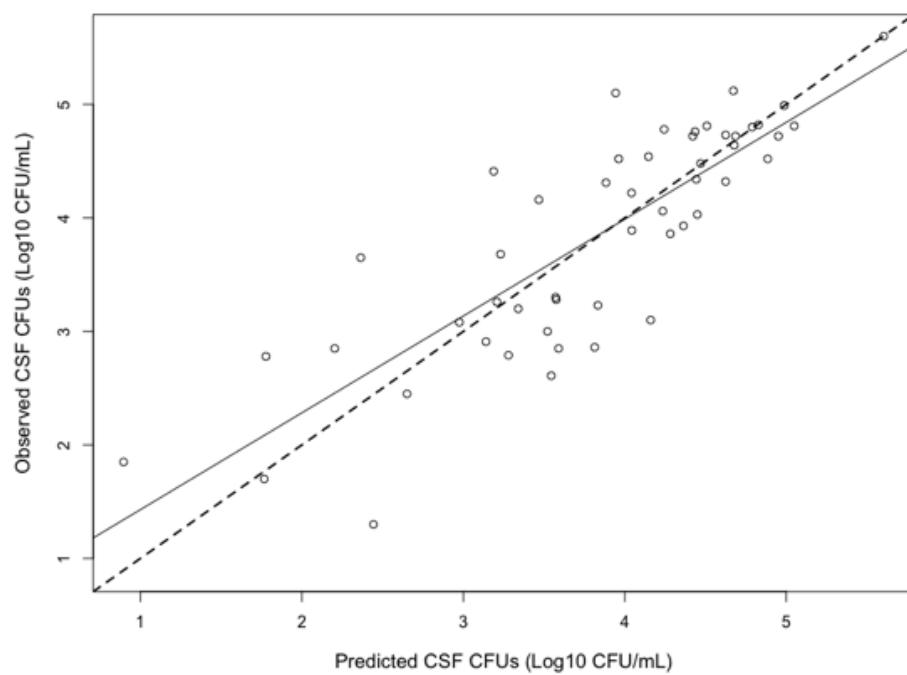
480

481

482 **FIG 2b**

483

484

485 **FIG 2c**

486

487 **Tables**

488

489 **TABLE 1** Mean and standard deviation observed isavuconazole concentrations
490 (mg/L) in the CNS by dose

	83.8 mg/kg	111.8 mg/kg
Brain	1.15 (± 1.5)	1.31 (± 0.96)
CSF	0.08 (± 0.049)	0.05 (± 0.028)
Ratio brain to plasma	0.69 (± 0.69)	0.42 (± 0.27)
Ratio CSF to plasma	0.044 (± 0.044)	0.019 (± 0.006)

491

492

493 **TABLE 2** Mean and standard deviation values for each parameter estimated
 494 from the rabbit population pharmacokinetic/pharmacodynamic-linked model

Parameter (units)	Mean	SD
Ka (h^{-1})	3.196	4.62
Cl/F (L/h)	2.639	2.26
Vc/F (L)	12.255	5.79
Vb/F (L)	165.876	51.75
Kcp (h^{-1})	17.942	9.82
Kpc (h^{-1})	20.135	9.83
Kcm (h^{-1})	13.959	13.52
Kmc (h^{-1})	16.593	9.00
Kgmax (\log_{10} CFU/mL)	0.027	0.02
Hg	13.670	9.13
C50g (mg/L)	1.754	1.61
IC (\log_{10} CFU/mL)	28,870.429	19,162.86
Kkmax (\log_{10} CFU/mL)	11.168	5.19
Popmax (\log_{10} CFU/mL)	409,294.206	449,097.00
Hk	3.697	2.66
C50k (mg/L)	1.928	1.42

495

496 **TABLE 3** Mean plasma and CSF AUC_{0–24} for each dose group and mean area
497 under the log₁₀ CFUs/mL over time of *Cryptococcus* spp. in the CSF

	83.8 mg/kg	111.8 mg/kg
Plasma (mg*h/L)	50.83 (±33.302)	99.83 (±31.292)
CSF (mg*h/L)	2.87 (±2.013)	1.35 (±0.598)
Log ₁₀ CFUs/mL versus time	1,304.36 (±240.36)	1,379.01 (±273.89)
Decline log ₁₀ CFU/mL	1.78 (±0.69)	1.89 (±0.33)

498

499

500 **TABLE 4** Plasma and CSF concentrations from two patients treated with
501 isavuconazonium sulfate

Dose	Serum (mg/L)	CSF (mg/L)	Ratio CSF/Plasma
Patient 1			
372 mg q24h	3.608	0.0296 ^a	0.008
1,116 mg q24h	11.936	0.0917 ^a	0.008
1,116 mg q24h	16.389	0.1312	0.008
744 mg q24h	13.924	0.0976 ^a	0.007
558 mg q24h	11.749	0.109	0.009
558 mg q24h	9.371	N/A	N/A
372 mg q24h	6.227	N/A	N/A
Patient 2			
372 mg q24h	4.489	0.0228 ^a	0.005
372 mg q24h	3.798	0.0405 ^a	0.011

502 ^aValues < 0.1 mg/L are extrapolated and are not actual measured drug

503 concentrations.

504 N/A, not available; q24h, every 24 h.

505 Adapted from Everson et al. (14).

